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ACCELERATED COMMUNICATION

α-Naphthoflavone Acts as an Antagonist of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin by Forming an Inactive Complex with the Ah Receptor

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SUMMARY

α-Naphthoflavone (ANF) has previously been shown to compete with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) for binding to the Ah receptor under conditions *in vitro*. However, ANF also prevents TCDD-elicited cytochrome P450IA1 induction, immunosuppression, and down-regulation of the estrogen receptor *in vivo* and within intact isolated cells. These data suggest that ANF is a TCDD antagonist. This study investigated the ability of ANF to transform the Ah receptor contained in rat hepatic cytosol or mouse hepatoma cells to a form that recognizes the dioxin-responsive enhancer element (DRE) upstream of the cytochrome P450IA1 gene. Gel retardation analysis indicated that TCDD- or β-naphthoflavone (BNF)-bound receptor was able to bind to the DRE, whereas essentially no receptor-DRE complexes were observed using cytosol incubated with ANF concentrations as high as 1000 nM. Furthermore, an excess of ANF, when added to cytosol just before TCDD, blocked, in a concentration-dependent manner, the ability of TCDD to transform the receptor to a form that bound to the DRE. These studies indicated that ANF binds to the receptor and confers on it a conformation that cannot recognize the DNA recognition sequence contained in the DRE. Although an excess of the agonist 2,3,7,8-tetrachlorodibenzofuran (TCDF) readily reversed the inhibitory actions of ANF, ANF was unable to reverse the effects of TCDD, TCDF, or BNF on the receptor. These studies suggested that TCDD binding, unlike that of ANF, results in a receptor conformation that has higher affinity for the ligand. Treatment of mouse hepatoma Hepa 1c1c7 cells with TCDD or BNF resulted in receptor contained in nuclear extracts that bound to the DRE. Only a very minor ligand-dependent protein-DNA complex was detected when cells were treated with ANF. These data indicated that ANF acts as an antagonist of TCDD by directly binding to the Ah receptor and eliciting a protein conformation that has very low affinity for DNA.

The specific and high affinity binding of TCDD and a number of related xenobiotics to a soluble intracellular protein, the Ah receptor, is the first step in a series of incompletely defined molecular events that lead to numerous biochemical, morphological, reproductive, and neoplastic effects in mammalian and nonmammalian organisms (1, 2). Evidence for the involvement of this protein in mediating these events comes from studies of genetic (3) and structure-activity relationships (4). The mechanisms involve a ligand-dependent alteration of the Ah receptor to a form that recognizes DNA in a sequence-specific manner and modulation of the expression (decreased or increased) of specific genes (5).

The most well studied gene modulated by TCDD is CYPIA1. The TCDD-Ah receptor complex binds to enhancer elements (DREs) upstream of the structural gene for CYPIA1 and induces its transcription (6–8). The core recognition sequence, 5′-T-GCGTG-3′ / 3′-A-CGCAC-5′, is present in four copies within the enhancer element (9). We have previously determined that the Ah receptor alone does not bind to this recognition sequence; the ligand-bound Ah receptor must interact with an additional protein to generate a heterodimer complex before recognition of the enhancer elements (10, 11). We have termed this second protein the Art protein. The exact molecular processes by which these events occur remain to be determined.

The polycyclic aromatic hydrocarbon BNF (Fig. 1) binds to the Ah receptor and can elicit the same pleiotropic responses in animals as observed with TCDD (12, 13). However, ANF (Fig. 1), an isomer of BNF, antagonizes the ability of TCDD to induce CYPIA1-associated enzyme activities (14, 15), immu-

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ABBREVIATIONS: TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; ANF, α-naphthoflavone; BNF, β-naphthoflavone; TCDF, 2,3,7,8-tetrachlorodibenzofuran; Art protein, Ah receptor-transforming protein; CYPIA1, cytochrome P450IA1; DRE, dioxin-responsive element; HSP 90, 90-kDa heat shock protein; DMSO, dimethyl sulfoxide; Ah, aryl hydrocarbon; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

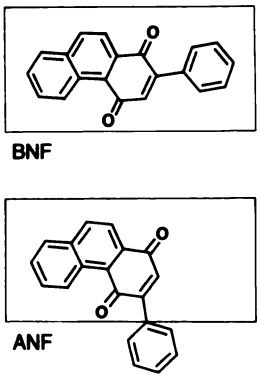

Fig. 1. Chemical structures of ANF and BNF. *Rectangles*, proposed 6.8- × 13.7-Å ligand binding site on the Ah receptor (40).

nosuppression (14), and down-regulation of the estrogen receptor (16). Earlier studies also noted that ANF inhibited the metabolism of selected carcinogens and their ability to inhibit proliferation of hamster ovary cells in culture (17). Because ANF also binds to the Ah receptor at the same site as TCDD and BNF (14), it is not clear whether this antagonism results from the formation of a ligand-receptor complex that does not bind to DNA, whether binding to nonspecific DNA is enhanced without the ability to recognize specific DNA sequences, or whether the complex binds to the DRE but in a manner that fails to regulate gene transcription. This study examined whether the ANF-Ah receptor complex binds to the DRE located upstream of the *CYPIA1* gene.

Materials and Methods

Chemicals. [1,6-^{3}H]TCDD (36 Ci/mmol) was obtained from ChemSyn (Lenexa, KS). Unlabeled TCDD was from Cambridge Isotope Laboratories (Woburn, MA). TCDF was obtained from the National Institute of Environmental Health Sciences (Research Triangle Park, NC). Unlabeled ANF and BNF were obtained from Sigma Chemical Co. (St. Louis, MO). [α-^{32}P]dATP (3000 Ci/mmol) was from Amersham Corp. (Arlington Heights, IL). Poly[d(I-C)] was purchased from Boehringer Mannheim (Indianapolis, IN). All other chemicals were as previously described (11).

Animals, tissue preparations, and incubations. Male Sprague-Dawley rats (175–200 g), from Charles River (Wilmington, MA), were housed under laboratory conditions for at least 3 days before use, with a 12-hr light cycle and free access to food and water. Hepatic cytosols (15–20 mg of protein/ml) were prepared in HEDG buffer [25 mM HEPES, pH 7.6 (20°), 1.5 mM EDTA, 1 mM dithiothreitol, 10% (v/v) glycerol], as described previously (18), and frozen at −80° until use. Procedures for cytosol incubations with [^{3}H]TCDD, with or without an excess of competing ligand, were as described previously (14, 18). Specific binding of [^{3}H]TCDD was assessed using the hydroxylapatite assay (19).

Assessment of DNA binding. The complementary oligodeoxyribonucleotides 5′-GATCCGGCTCTTCTCACGCAACTCCGAGCTCA-3′ and 5′-GATCTGAGCTCGGAGTTGCGTGAGAAGAGCCG′-3′ were synthesized and ^{32}P-labeled at the 5′ ends, as described (8, 11). These sequences, when annealed, contain a single core recognition sequence (underlined) for the DNA-binding form of the Ah receptor (8). Cytosolic protein (90 μg) was mixed with 200 ng of poly[d(I-C)] and approximately 3–5 × 10^4 cpm of the DNA probe (specific activity, 10^8 cpm/μg of DNA), for 20 min at 20°, and was analyzed by nondenaturing gel electrophoresis, as described previously (8). For the analysis of nuclear extracts, 5 μg of protein and 1.2 μg of poly[d(I-C)] were used. The Ah receptor-^{32}P-DRE complexes were quantified by isolating the TCDD-dependent bands, measuring the amount of ^{32}P contained in these bands by liquid scintillation counting, and subtracting the amount of radioactivity in lanes with samples containing no TCDD.

Cell culture and preparation of nuclear extracts. Mouse hepatoma cells (Hepa 1c1c7) were obtained from Dr. James P. Whitlock, Jr. (Stanford University), and cultured at 37°, in 100- × 20-mm dishes, with Eagle's modified minimal essential medium (Sigma) containing 10% fetal bovine serum (GIBCO, Grand Island, NY) and 20 μg gentamycin/ml. Confluent plates were layered with 2.5 ml of serum-free medium and treated with TCDD, BNF, or ANF contained in DMSO. The final DMSO concentration was 4 μl/ml of medium, and 10–20 plates were used for each compound. The cells were incubated at 37° for 1 hr, medium was removed, plates were scraped, and cells were collected at 4° in Hanks' balanced salt solution. The cell pellets (1100 rpm, 7 min, 4°) were resuspended in 2 volumes of HEDG buffer and homogenized with a Teflon pestle. Nuclei were prepared as described previously (20), resuspended in 4 ml of HEDG buffer containing 0.55 M KCl, and allowed to incubate at 4° for 1 hr, to extract nuclear protein. After centrifugation at 105,000 × *g* for 1 hr, the protein and final salt concentrations (0.46–0.49 M) of the extracts were determined as previously described (18, 20).

Results

Binding of TCDD-, BNF-, and ANF-bound receptor to the DRE. As previously determined (11), gel retardation analysis of cytosol incubated with or without 2 nM TCDD indicated the presence of a single TCDD-dependent protein-DRE complex (Fig. 2, compare *lanes 1* and *2*). A protein-DRE complex having the same mobility was also observed using BNF concentrations of 1–1000 nM (Fig. 2, *lanes 4–7*). In contrast, essentially no receptor-DRE complexes were observed using ANF concentrations as high as 1000 nM (Fig. 2, *lanes 8–12*). (A minor and diffuse ANF-dependent band was observed at ANF concentrations of >100 nM. However, this band had a slightly slower mobility than the TCDD- and BNF-dependent band.)

We previously determined that both BNF and ANF inhibited TCDD binding to the Ah receptor contained in rat hepatic cytosol, in a competitive manner; the relative affinities of the

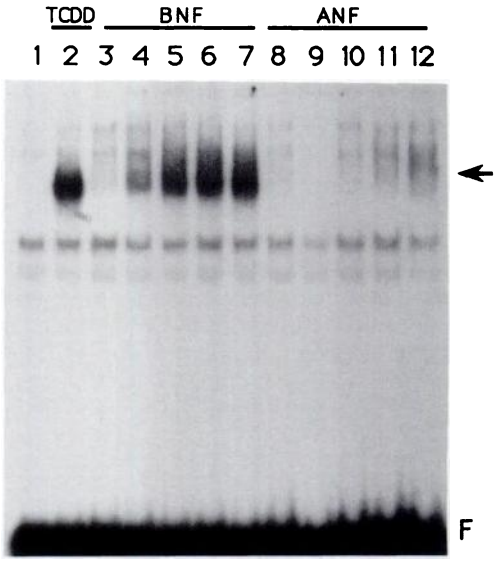

Fig. 2. Gel retardation analysis of cytosol incubated with TCDD, BNF, or ANF. Cytosols were treated with ligands for 2 hr at 20° before the addition of poly[d(I-C)] and ^{32}P-labeled DRE, as follows: *lanes 1* and *2*, absence or presence, respectively, of 2 nM TCDD; *lanes 3–7*, presence of 0.1, 1.0, 10, 100, or 1000 nM BNF; *lanes 8–12*, presence of 0.1, 1.0, 10, 100, or 1000 nM ANF. *Arrow*, position of the agonist (TCDD, BNF)-dependent band; *F*, free ^{32}P-labeled DRE.

receptor for these ligands were approximately 50- and 500-fold less, respectively, than for TCDD (14). ANF, when added to cytosol just before TCDD, blocked, in a concentration-dependent manner, the ability of TCDD to transform the receptor to a form that bound to the DRE (Fig. 3A). Using a TCDD concentration of 2 nM, nearly complete blockage was observed at 1000 nM ANF when the coincubation was carried out at 20° for 2 hr. There was also good agreement between loss of TCDD-dependent DRE binding, when quantitated, and loss of [^{3}H]TCDD specific binding, as determined by the hydroxylapatite assay (Fig. 3B). Together, these studies indicated that ANF binds to the Ah receptor and confers on it a conformation that cannot recognize the DNA recognition sequence contained in the DRE.

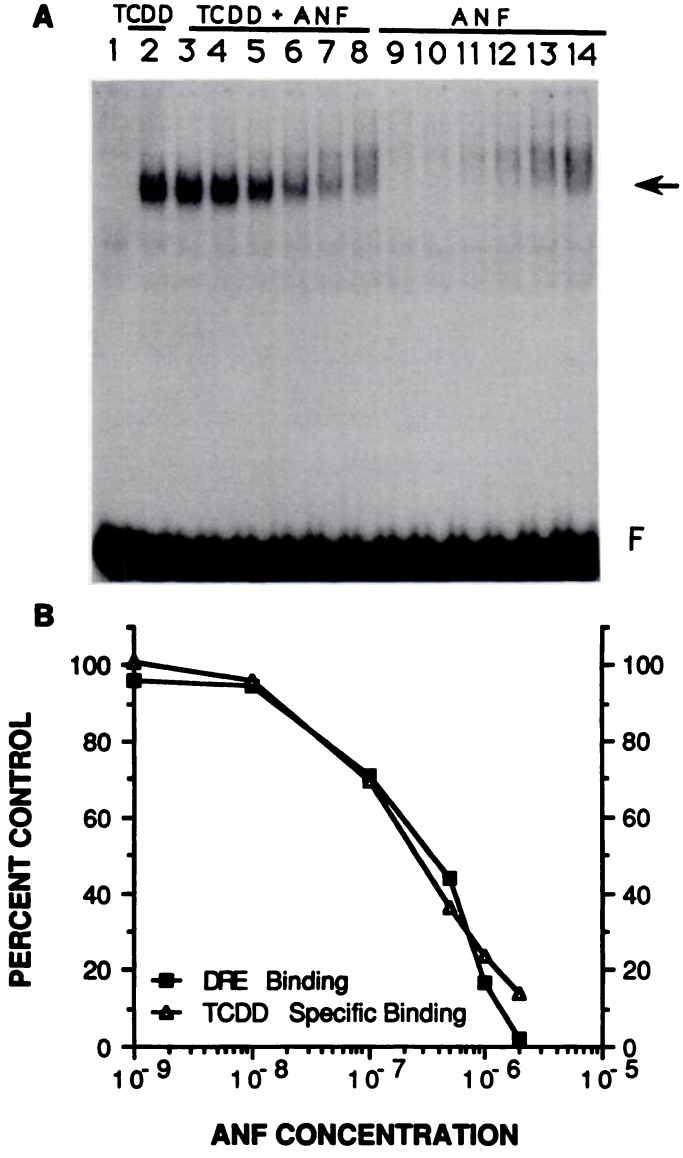

Fig. 3. A, Gel retardation analysis of cytosol coincubated with TCDD and various concentrations of ANF. Cytosols were treated for 2 hr at 20°, in the absence or presence of 2 nM [^{3}H]TCDD (*lanes 1* and *2*, respectively) or in the presence of 2 nM [^{3}H]TCDD plus 1.0, 10, 100, 500, 1000, or 2000 nM ANF (*lanes 3–8*), before the addition of poly[d(I-C)] and ^{32}P-labeled DRE. *Lanes 9–14*, cytosol incubated with 1.0, 10, 100, 500, 1000, or 2000 nM ANF. B, Quantitation of loss of Ah receptor-DRE binding (■), shown in A, and [^{3}H]TCDD specific binding (Δ), as determined by the hydroxylapatite assay. Results are presented as percentage of control cytosol, which was incubated with [^{3}H]TCDD in the absence of ANF.

Reversibility of ANF and TCDD actions on the Ah receptor. We determined whether the TCDD-elicited transformation of the Ah receptor was reversible by ANF. Cytosol was incubated with 1 nM TCDD for 2 hr at 20°, which has been found to give maximal transformation of the Ah receptor to a DRE-binding state (21). After this incubation, ANF (1000 nM) was added and DRE binding was determined at various times up to 4 hr. Under these conditions, ANF was unable to reverse the effects of TCDD on the receptor (Fig. 4A). Essentially the same study was performed using BNF as the agonist. Similarly, ANF (1000 nM) was unable to reverse the effects of 10 nM BNF on the receptor (data not shown). Thus, the irreversible nature of the transformed agonist-receptor complex was not dependent on the relative affinity of the particular ligand used as the agonist.

In a similar manner, we sought to determine whether the antagonistic actions of ANF on the receptor were reversible by agonist. Because a high concentration of ANF (1000 nM) was used to ensure complete saturation of the receptor, and the aqueous solubility of TCDD is limited, we chose to use the agonist TCDF to reverse the effect of ANF. TCDF has nearly the same affinity as TCDD for the receptor contained in rat hepatic cytosol (22), and TCDF-elicited transformation of the receptor was not reversible by ANF (Fig. 4A, *lanes 8* and *9*). Within 4 hr, 500 nM TCDF nearly completely reversed the inhibitory action of ANF (Fig. 4B). We interpret these studies to indicate that binding of the agonists TCDD, TCDF, or BNF to the receptor results in the transformation of this protein to a ligand-bound complex that is not readily reversible under conditions *in vitro*; such a transformation does not occur after ANF binding.

Ability of ANF to form a DNA-binding receptor complex in Hepa 1c1c7 cells. The experiments described above were performed under *in vitro* conditions using only cytosolic protein. We tested whether ANF has the ability to transform the Ah receptor complex contained in intact cells to a form that localizes to the nucleus. Hepa 1c1c7 cells have been shown to increase transcription of the *CYPIA1* gene in response to TCDD (23) and possess the same transcriptional promoter as that found for this gene in C57BL/6 mice (24). As previously shown (9), treatment of these cells with 2 nM TCDD resulted in the presence, in nuclear extracts, of receptor that binds to the ^{32}P-labeled DRE (Fig. 5, compare *lanes 1* and *2*). This protein-DNA complex has the same mobility as that found in rat hepatic cytosol incubated with TCDD (Fig. 2). Treatment of cells with BNF yielded the same result (Fig. 5, *lane 4*). However, only a very faint ligand-dependent band was observed when cells were incubated with an ANF concentration of 1000 nM (Fig. 5, *lane 3*). These results, demonstrating that within intact hepatoma cells ANF binds to the Ah receptor and forms a complex with only very weak DRE-binding ability, are consistent with the studies using receptor contained in crude cytosol and those previous investigations showing antagonism of TCDD-elicited effects *in vivo* (14, 15) and in isolated cells (16).

Discussion

These data, combined with results previously presented (14, 15), indicate that ANF acts as an antagonist of TCDD by

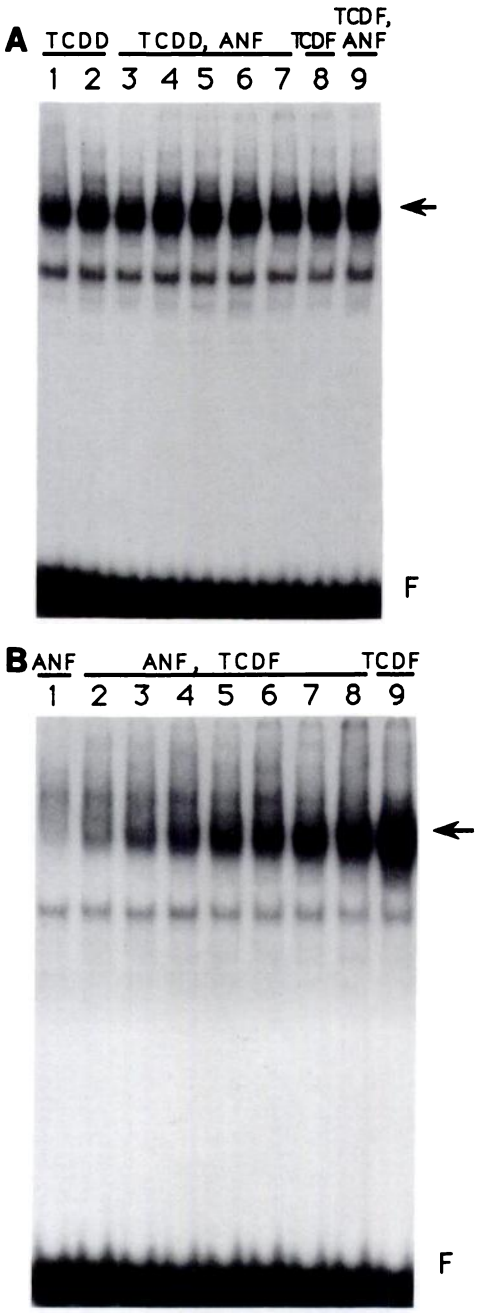

Fig. 4. Gel retardation analysis to determine the relative reversibility of ANF- and TCDD-Ah receptor complexes. A, Reversibility of TCDD- and TCDF-elicited Ah receptor transformation by ANF. *Lanes 1* and *2*, cytosol incubated with TCDD (1 nM) for 2 hr at 20° and then for an additional 4 hr at either 20° or 0°, respectively. *Lanes 3–7*, cytosol incubated with TCDD for 2 hr at 20° and then for various times (0.16, 0.5, 1, 2, or 4 hr) in the presence of 1000 nM ANF. *Lane 8*, cytosol incubated with TCDF (10 nM) for 6 hr at 20°. *Lane 9*, cytosol incubated with TCDF for 2 hr at 20° and then for an additional 4 hr in the presence of 1000 nM ANF. B, Reversibility by TCDF of ANF-elicited inhibition of transformation. *Lane 1*, cytosol incubated with ANF (1000 nM) for 2 hr at 20°. *Lanes 2–8*, cytosol incubated with ANF for 2 hr at 20° and then for various times (0.16, 0.33, 0.5, 0.75, 1.0, 2.0, or 4 hr) in the presence of 500 nM TCDF. *Lane 9*, cytosol incubated for 6 hr in the presence of TCDF only. All of the aforementioned steps were performed before the addition of poly[d(I-C)] and ^{32}P-labeled DRE.

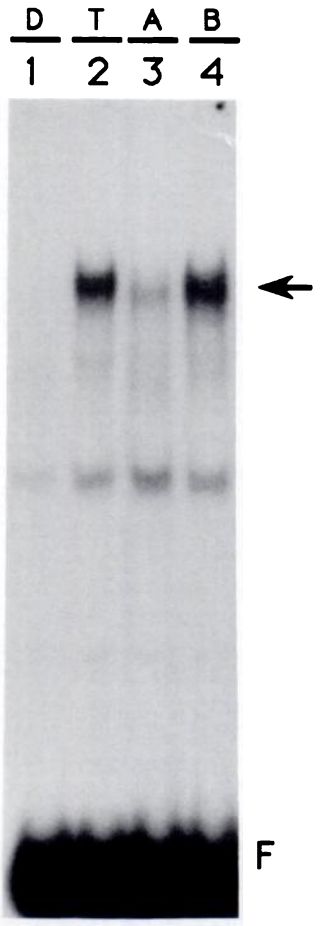

Fig. 5. Gel retardation analysis of nuclear extracts prepared from Hepa 1c1c7 cells treated with TCDD, ANF, or BNF. Cells were incubated with vehicle (DMSO, 4 μl/ml) (*D*) (*lane 1*), 2 nM TCDD (*T*) (*lane 2*), 1000 nM ANF (*A*) (*lane 3*), or 1000 nM BNF (*B*) (*lane 4*) for 1 hr at 37° before harvesting and preparation of nuclear extracts and the determination of DRE binding. *Arrow*, TCDD/BNF-dependent protein-DNA complex.

directly binding to the Ah receptor and eliciting a conformation that has only a very low affinity for DNA. To our knowledge, this is the first time that a ligand that binds to the Ah receptor has been found not to transform the receptor to a form that demonstrates high affinity and specific DNA binding. Thus, these data, and the fact that this ligand, ANF, also antagonizes TCDD-like activity *in vivo*, corroborate the relationship between Ah receptor presence and its function, in this case to act as a *trans*-acting element to enhance the transcription of the *CYP1A1* gene.

Based on our data and those of others, we have previously proposed a model for the transformation of the Ah receptor to a form that binds DNA (25). The unoccupied receptor exists as an oligomeric complex (~300 kDa) composed of the monomeric Ah receptor protein (~100 kDa), a dimer of HSP 90, and possibly an as yet uncharacterized protein of ~50 kDa (26–28). Agonist (i.e., TCDD) binding to the receptor results in dissociation of the oligomeric complex and the exposure of a domain on the ligand-binding receptor that then is able to interact specifically with another protein, the Art protein. The formation of the heterodimer results in a structural conformation that has both high affinity and specificity for the DRE (11). Previous studies have also suggested that transformation of the Ah receptor to the heterodimer form that binds to the DRE

entails stabilization of the ligand-receptor complex (29–31). Our present finding that, once transformed, the TCDD-, TCDF-, or BNF-receptor complex is not readily reversible, even in the presence of a large excess of competing ANF, is consistent with this.

The question then remains as to how ANF prevents transformation to a form that binds DNA. Although our present results do not address this directly, the data are consistent with a hypothesis that ANF binding to the Ah receptor results in a conformation of the receptor that cannot interact with the Art protein, i.e., the formation of the transcriptionally active heterodimer complex is prevented. ANF-induced inhibition of the DRE-binding complex is readily reversible by the agonist TCDF, whereas TCDD-, TCDF-, and BNF-elicited transformations are not reversible by ANF. This suggests that heterodimer formation and stabilization of ligand binding do not occur when the receptor is bound with ANF. We have previously determined that, 1) under conditions where transformation of the TCDD-receptor complex to the heteromeric form is inhibited, the relative percentage of the specific binding existing as the ligand-bound monomeric Ah receptor is very high (>40%) (32) and 2) TCDD binding is also not readily reversible from the partially purified monomeric Ah receptor form.[1] Together, these data suggest that a step before heterodimer formation is responsible for ligand-binding stabilization and, further, imply that ANF acts by binding to the Ah receptor but not promoting Ah receptor-HSP 90 dissociation. Such a mechanism has been indicated for the antisteroidal action of the antiglucocorticoid RU38486 and the antiprogestin RU486. Both have been shown to stabilize the interaction of their respective steroid hormone receptor with HSP 90, thus preventing specific responsive element binding (33, 34). Work is presently underway in our laboratory to examine this possibility with the ANF-Ah receptor complex.

The formation of a ligand-Ah receptor complex that does not undergo further transformation to a DNA-binding form may be only one of the mechanisms whereby compounds act as TCDD antagonists. To date, a number of structurally related chemicals have been reported to antagonize some of the biological and toxicological effects of TCDD. These include 2,2′,4,4′,5,5′-hexachlorobiphenyl (35), 1-amino-3,7,8-trichlorodibenzo-*p*-dioxin (36, 37), and 6-methyl-1,3,8-trichlorodibenzofuran (38). It is of particular interest that the ^{125}I-analog of the latter compound has been shown to bind to the Ah receptor contained in rat hepatoma cells, to form a complex that localizes to the nuclear fraction and binds to the DRE but apparently fails to activate *CYPIA1* gene transcription (38).

Examination of the constraints for ligand binding, especially as they relate to the differences between the molecular structures of ANF and BNF, may prove extremely useful in determining the exact mechanism whereby ANF binding inhibits receptor transformation. The agonist BNF, a planar molecule (39), fits the structural requirements for the binding of a variety of compounds to the Ah receptor (40) (see also Fig. 1). The binding of molecules that have a good fit to the 6.8- × 13.7-Å envelope that has been proposed to describe the ligand binding site results in dissociation of HSP 90 and formation of the transcriptionally active heterodimer complex, by mechanisms that are not precisely defined. However, ANF is more stable in a nonplanar and angular configuration (39) and offers a poor fit to this site (40) (Fig. 1). In this case, the filling of only a half-site by ANF may result in the trapping of the receptor in a configuration that is not able to undergo the conformational and/or biochemical changes that are necessary for further transformation. Regardless of the final mechanisms involved, it is clear from these studies that ANF will serve as an extremely useful tool not only to examine the functional relationships between receptor action and altered gene expression but also to dissect the ligand and protein interactions and structural requirements necessary for the steps involved in Ah receptor transformation.

[1]E. C. Henry and T. A. Gasiewicz, unpublished observations.

Acknowledgments

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References

1. Poland, A., and J. Knutson. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity. *Annu. Rev. Pharmacol. Toxicol.* **22**:517–554 (1982).
2. Gasiewicz, T. A. Nitro compounds and related phenolic pesticides, in *Handbook of Pesticide Toxicology. Classes of Pesticides* (W. J. Hayes, Jr., and E. R. Laws, Jr., eds.), Vol. 3. Academic Press, Inc., New York, 1191–1269 (1991).
3. Poland, A., and E. Glover. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin: segregation of toxicity with the *Ah* locus. *Mol. Pharmacol.* **17**:86–94 (1980).
4. Safe, S. Comparative toxicology and mechanism of action of polychlorinated dibenzo-*p*-dioxins and dibenzofurans. *Annu. Rev. Pharmacol. Toxicol.* **26**:371–399 (1986).
5. Whitlock, J. P., Jr. Genetic and molecular aspects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin action. *Annu. Rev. Pharmacol. Toxicol.* **30**:251–277 (1990).
6. Jones, P. B. C., D. R. Galeazzi, J. M. Fisher, and J. P. Whitlock, Jr. Control of cytochrome P_1-450 gene expression by dioxin. *Science (Washington D. C.)* **227**:1499–1502 (1985).
7. Fujisawa-Sehara, A., K. Sogawa, C. Nishi, and Y. Fujii-Kuriyama. Regulatory DNA elements localized remotely upstream from the drug-metabolizing cytochrome P450 gene. *Nucleic Acids Res.* **14**:1465–1477 (1986).
8. Denison, M. S., J. M. Fisher, and J. P. Whitlock, Jr. Inducible receptor-dependent protein-DNA interactions at a dioxin-responsive transcriptional enhancer. *Proc. Natl. Acad. Sci. USA* **85**:2528–2532 (1988).
9. Denison, M. S., J. M. Fisher, and J. P. Whitlock, Jr. Protein-DNA interactions at recognition sites for the dioxin-Ah receptor complex. *J. Biol. Chem.* **264**:16478–16482 (1989).
10. Elferink, C. J., T. A. Gasiewicz, and J. P. Whitlock, Jr. Protein-DNA interactions at a dioxin-responsive enhancer: evidence that the transformed Ah receptor is heteromeric. *J. Biol. Chem.* **265**:20708–20712 (1990).
11. Gasiewicz, T. A., C. J. Elferink, and E. C. Henry. Characterization of multiple forms of the Ah receptor: recognition of a dioxin-responsive enhancer involves heteromer formation. *Biochemistry* **30**:2909–2916 (1991).
12. Poland, A., E. Glover, and A. Kende. Stereospecific, high affinity binding of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin by hepatic cytosol: evidence that the binding species is receptor for induction of aryl hydrocarbon hydroxylase. *J. Biol. Chem.* **215**:4936–4946 (1976).
13. Silkworth, J. B., L. Antrim, and L. S. Kaminsky. Correlations between polychlorinated biphenyl immunotoxicity, the aromatic hydrocarbon locus, and liver microsomal enzyme induction in C57BL/6 and DBA/2 mice. *Toxicol. Appl. Pharmacol.* **75**:156–165 (1984).
14. Blank, J. A., A. N. Tucker, J. Sweatlock, T. A. Gasiewicz, and M. I. Luster. α-Naphthoflavone antagonism of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced murine lymphocyte ethoxyresorufin-*O*-deethylase activity and immunosuppression. *Mol. Pharmacol.* **32**:168–172 (1987).
15. Merchant, M., L. Arellano, and S. Safe. The mechanism of action of α-naphthoflavone as an inhibitor of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced *CYPIA1* gene expression. *Arch. Biochem. Biophys.* **281**:84–89 (1990).
16. Merchant, M., V. Krishnan, and S. Safe. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) as an antiestrogen in MCF-7 human breast cancer cells: antagonism by α-naphthoflavone (αNF). *Toxicologist* **11**:A19 (1991).
17. Diamond, L., and H. V. Gelboin. *Alpha*-naphthoflavone: an inhibitor of hydrocarbon cytotoxicity and microsomal hydroxylase. *Science (Washington D. C.)* **166**:1023–1025 (1969).
18. Gasiewicz, T. A., and P. A. Bauman. Heterogeneity of the rat hepatic Ah receptor and evidence for transformation *in vitro* and *in vivo*. *J. Biol. Chem.* **262**:2116–2120 (1987).
19. Gasiewicz, T. A., and R. A. Neal. The examination and quantitation of tissue cytosolic receptors for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin using hydroxylapatite. *Anal. Biochem.* **124**:1–11 (1982).
20. Henry, E. C., G. Rucci, and T. A. Gasiewicz. Characterization of multiple forms of the Ah receptor: comparison of species and tissues. *Biochemistry* **28**:6430–6440 (1989).

21. Denison, M. S., and E. F. Yao. Characterization of the interaction of transformed rat hepatic cytosolic Ah receptor with a dioxin responsive transcriptional enhancer. *Arch. Biochem. Biophys.* **284**:158–166 (1990).
22. Gasiewicz, T. A., and G. Rucci. Cytosolic receptor for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: evidence for a homologous nature among various mammalian species. *Mol. Pharmacol.* **26**:90–98 (1984).
23. Miller, A., D. Israel, and J. P. Whitlock, Jr. Biochemical and genetic analysis of variant mouse hepatoma cells defective in the induction of benzo[*a*]pyrene-metabolizing enzyme activity. *J. Biol. Chem.* **258**:3523–3527 (1983).
24. Hirst, M. A., K. W. Jones, and J. P. Whitlock, Jr. Activation of cytochrome P450IA1 gene expression by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in wild-type and high-activity variant mouse hepatoma cells. *Mol. Carcinogenesis* **2**:40–46 (1989).
25. Gasiewicz, T. A., and E. C. Henry. Different forms of Ah receptor: possible role in species- and tissue-specific responses to TCDD, in *Biological Basis for Risk Assessment of Dioxins and Related Compounds* (R. J. Scheuplein, K. L. Burgess, M. Gallo, and C. A. van der Heijden, eds.), Banbury Report 35. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, in press (1991).
26. Perdew, G. H. Chemical cross-linking of the cytosolic and nuclear forms of the Ah receptor: determination of protein composition. *Toxicologist* **11**:A12 (1991).
27. Denis, M., L. Poellinger, A.-C. Wikstrom, and J.-A. Gustafsson. Association of the dioxin receptor with the M_r 90,000 heat shock protein: a structural kinship with the glucocorticoid receptor. *Biochem. Biophys. Res. Commun.* **155**:801–807 (1988).
28. Perdew, G. H. Association of the Ah receptor with the 90-kDa heat shock protein. *J. Biol. Chem.* **263**:13802–13805 (1988).
29. Kester, J. E. *Physicochemical Characterization of the Rat Hepatic Ah Receptor and Its Interaction with 2,3,7,8-Tetrachlorodibenzo-p-dioxin.* Ph.D. Thesis, The University of Rochester, Rochester, NY (1987).
30. Farrell, K., and S. Safe. Absence of positive co-operativity in the binding of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin to its cytosolic receptor protein. *Biochem. J.* **244**:539–546 (1987).
31. Rucci, G., and T. A. Gasiewicz. *In vivo* kinetics and DNA-binding properties of the *Ah* receptor in the golden Syrian hamster. *Arch. Biochem. Biophys.* **265**:197–207 (1988).
32. Henry, E. C., and T. A. Gasiewicz. Inhibition and reconstitution of Ah receptor transformation *in vitro:* role and partial characterization of cytosolic factor(s). *Arch. Biochem. Biophys.* **288**: 149–156 (1991).
33. Segnitz, B., and U. Gehring. Mechanism of action of a steroidal antiglucocorticoid in lymphoid cells. *J. Biol. Chem.* **265**:2789–2796 (1990).
34. Renoir, J.-M., C. Radanyi, I. Jung-Testas, L. E. Faber, and E.-E. Baulieu. The nonactivated progesterone receptor is a nuclear heterooligomer. *J. Biol. Chem.* **265**:14402–14406 (1990).
35. Biegel, L., M. Harris, D. Davis, R. Rosengren, L. Safe, and S. Safe. 2,2′4,4′,5,5′-Hexachlorobiphenyl as a 2,3,7,8-tetrachlorodibenzo-*p*-dioxin antagonist in C57BL/6J mice. *Toxicol. Appl. Pharmacol.* **97**:561–571 (1989).
36. Luster, M. I., L. H. Hong, R. Osborne, J. A. Blank, G. Clark, M. T. Silver, G. A. Boorman, and W. F. Greenlee. 1-Amino-3,7,8-trichlorodibenzo-*p*-dioxin: a specific antagonist for TCDD-induced myelotoxicity. *Biochem. Biophys. Res. Commun.* **139**:747–756 (1986).
37. Hong, L. H., J. D. McKinney, and M. I. Luster. Modulation of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-mediated myelotoxicity by thyroid hormones. *Biochem. Pharmacol.* **36**:1361–1365 (1987).
38. Piskorska-Pliszczynzka, J., B. Astroff, T. Zacharewski, M. Harris, R. Rosengren, V. Morrison, L. Safe, and S. Safe. Mechanism of action of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin antagonists: characteristics of 6-[^{125}I]methyl-8-iodo-1,3-dichlorodibenzofuran-Ah receptor complexes. *Arch. Biochem. Biophys.* **284**:193–200 (1991).
39. Rossi, M., J. S. Cantrell, A. J. Farber, T. Dyott, H. L. Carrell, and J. P. Glusker. Molecular structures of 5,6- and 7,8-benzoflavones, inhibitors of aryl hydrocarbon hydroxylase. *Cancer Res.* **40**:2774–2784 (1980).
40. Gillner, M., J. Bergman, C. Cambillau, B. Fernstrom, and J.-A. Gustafsson. Interactions of indoles with specific binding sites for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rat liver. *Mol. Pharmacol.* **28**:357–363 (1985).

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